Solubility and extractability in the Pharmaceutical Sciences: A demonstration to address these essential concepts

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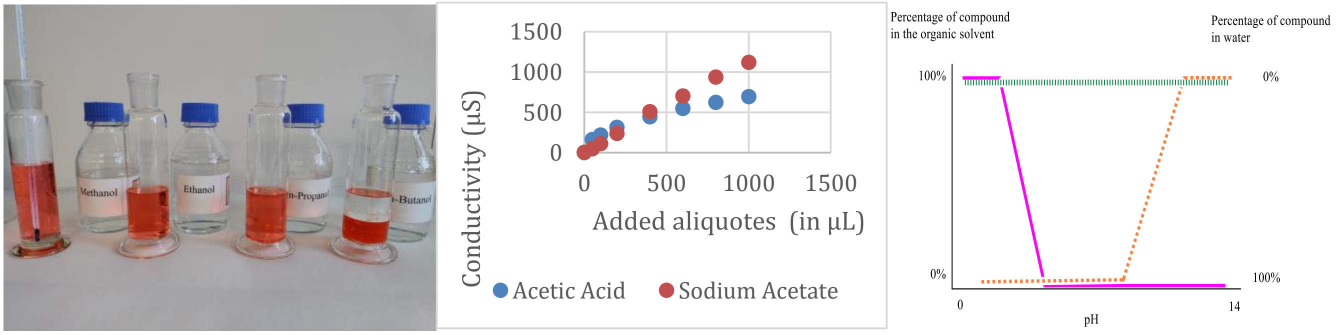
ABSTRACT

Understanding the solubility and extractability of organic compounds plays a crucial role in the pharmaceutical and biomedical fields. It is essential to learn these concepts early in the course curriculum. The aim of this demonstration is to help students understand the basic principles of solubility and extractability in a practical, interactive, and interesting way. This is accomplished with the help of a series of simple experiments in which common reagents and molecules will be used. The demonstration covers three main topics. The first part is designed to explain the intrinsic solubility in water. This solubility will depend on a number of properties, including their ratio of polar/nonpolar groups and their ability to form hydrogen bonds. The second part investigates potential ionization in water using results obtained from conductimetry experiments. The third part is related to the concept of extractability by presenting the results obtained for pH-dependent extractions of representative compounds from an aqueous medium with an organic medium. A quantification of the extracted compound at each pH is performed after the evaporation of the organic solvent. Extractability curves are then drawn, using the weights obtained after evaporation, and discussed.

GRAPHICAL ABSTRACT



Solubility and H bonds

Solubility and ionization

Extractability

KEYWORDS
Second-Year Undergraduate, Organic Chemistry, Hands-On Learning/Manipulatives, Acids/Bases, Organic compounds, pKa, Solubility, Extractability

INTRODUCTION

The solubility and extractability of organic compounds are different depending on their nature. A compound may be soluble in an organic solvent or an aqueous medium but is not necessarily extractable by it and vice versa. Variations in pH may also influence the solubility and extractability. Understanding and applying these two fundamental concepts are challenges faced within the pharmaceutical field for the preparation of drugs in either research or therapeutics. In theory and in practice, these concepts are known to be difficult to understand and to apply.[1] Therefore, a live demonstration was conducted to facilitate the comprehension of these concepts for second-year undergraduate students in Pharmaceutical Sciences.

Water solubility plays an essential role in the pharmaceutical field as most medications are in contact with an aqueous environment. This is because the human body, being primarily composed of water, relies on aqueous solutions for the absorption and distribution of drugs. Drugs that possess the right hydro/lipophilic profile are able to cross biological membranes, including those that separate different compartments within cells. During the live demonstration, these lipid bilayers are represented by the organic solvent, while the cytosolic compartments are represented by water. Hence, a thorough understanding of a drug's solubility in water is essential to be able to later predict its behavior within the body and to ensure an effective therapeutic outcome.

For many years, this demonstration has shown, through a combination of simple experiments, the differences that exist between solubility and extractability by using commonly used molecules in the pharmaceutical field. This session lasted approximately 1 h in the presence of a group of 30 students. The main experiments presented are reported below, and additional assays are in the Supporting Information. During the covid19 pandemic, a video was recorded and proposed as an alternative to the live presentation.

One of the main goals of this demonstration was to illustrate the difference between functional groups that possess a hydroxyl group. During the organic chemistry course, functional groups such as aliphatic alcohols, phenol (or aromatic hydroxyl), and carboxylic acids are presented as acid entities. In other words, the hydrogen of the functional group is mobile so the group can be deprotonated. Herein, the aim of the demonstration is to clearly show that these functional groups have a different behavior in the aqueous medium or in the biphasic media due to the different electronic surroundings, which will modify their acid/base properties. Compounds with a nitrogen functional group are also included in these assays.

Unlike many reports that are dedicated to teaching the extraction of compounds and their solubility,[2-10] the originality of this demonstration is based on the combination of three approaches to fully integrate the concepts of solubility and extractability of pure organic compounds. This demonstration is also unique in the fact that it shows the ionization phenomenon by using conductivity measures and helps assimilate the extractability concept through quantification of the compound present in the organic phases by evaporating the corresponding phases.

DESIGN OF EXPERIMENTS

Part I: Intrinsic solubility

First, to explore how various substances interact with water, particularly their ability to dissolve or mix in it, a series of short-chain alcohols and polyhydroxylated compounds are tested. The experiment aims to understand how the balance between polar and nonpolar regions in a molecule affects its solubility in water.

Second, the influence of a molecule’s hydrogen bonds on its solubility will be examined by using two assays. The first assay will involve mixing alcohol and water, which modifies hydrogen bond interactions. This modification is observed by measuring the temperature of the mixture. The second assay, on the other hand, will involve mixing 1-propanol with an aqueous sodium chloride solution, which will induce a competition between the two in forming hydrogen bonds.

Third, to demonstrate how the ionized state of a molecule impacts its solubility, an assay showing the behavior of carboxylic acids (both lipophilic and hydrophilic) and their corresponding sodium salts in water was conducted.

Part II: The potential ionization when solubilized in water

To fully understand a molecule’s solubility in water, its ionized state is measured using conductimetry. The appearance of a current indicates the presence of ions in the solution. Its relative significance will be determined by comparing the results using sodium chloride as a reference. In this assay, functional groups such as alcohol, carboxylic acid and carboxylate, amine, and quaternary ammonium will be used as a reference and tested. For practical reasons, the results alone are presented during the live demonstration.

Part III: Extractability

The experiments were performed by using a liquid-liquid extraction procedure before the live demonstration. The aqueous phases used were buffered solutions at specific pHs, while the organic solvent used was diethyl ether. After mixing both phases, the organic phase was collected, and the solvent was removed under reduced pressure. Any potential residue was quantified. The results are expressed in weight of the residue or in percentage of recovery and were reported in a graph. These extractability curves, representing the class of compounds, such as lipophilic acids, phenol, alcohol, and an aromatic amine, were presented and discussed during the live demonstration.

EXPERIMENTS

1. INTRINSIC SOLUBILITY.

1.1. The influence of the hydrophilicity and the lipophilicity.

To show the impact of the polar/nonpolar groups ratio within a molecule on water solubility, a series of short-chain alcohols (methanol, ethanol, 1-propanol, and 1-butanol) was used (Figure 1). Throughout the figures of the manuscript, in order to better understand the ability of molecules to form different intermolecular interactions involved in the solubility process, the groups in the chemical structures are highlighted (**in red for the polar groups interacting by H bonds**, **in blue for the polar groups interacting by ionic bonds**, **in green for the hydrophobic nonpolar groups**). This will help to show the students that the percentage of the polar group in these alcohols is reduced when the size of the alkyl group increases (Figure 1).

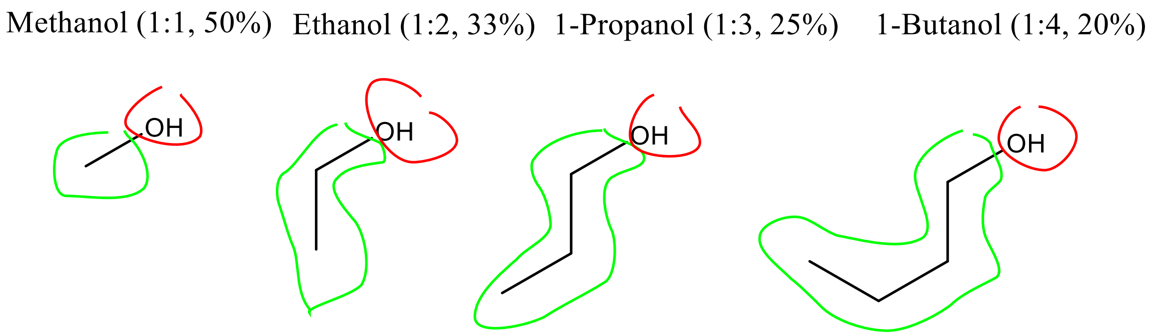


Figure 1. Chemical structures of the alcohols used to show the evolution of water solubility. In parentheses, the ratio of the number of polar groups to the number of nonpolar groups (in red, the polar group interacting by H bonds; in green, the hydrophobic nonpolar group) followed by the corresponding percentage of the polar group.

An equal volume of each alcohol and demineralized water are mixed. To facilitate visualization of the phenomenon, water is dyed with E124. The results of this assay are shown in Figure 2. For the first three members of the series, a homogeneous mixture is obtained immediately.

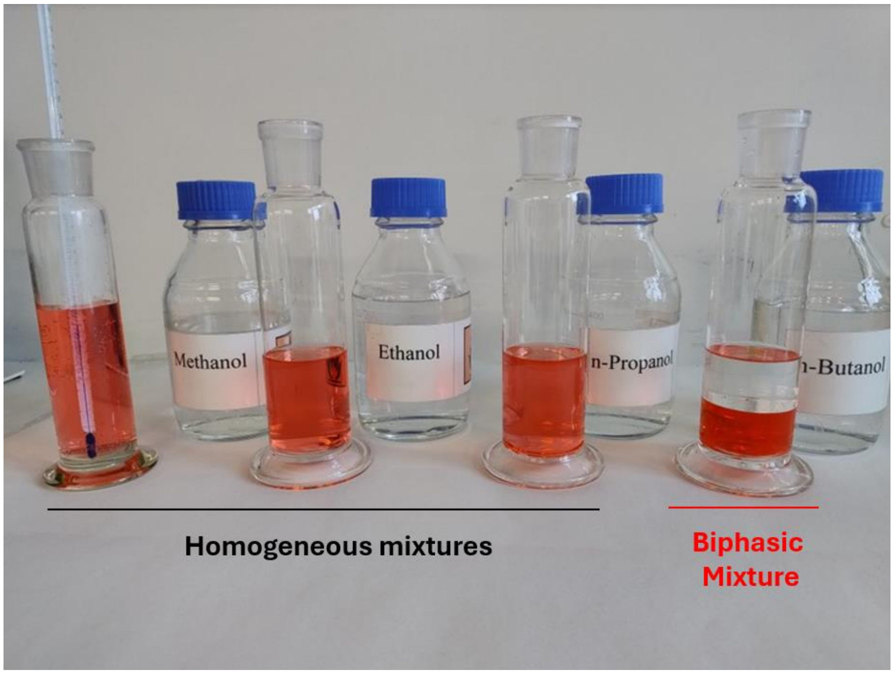
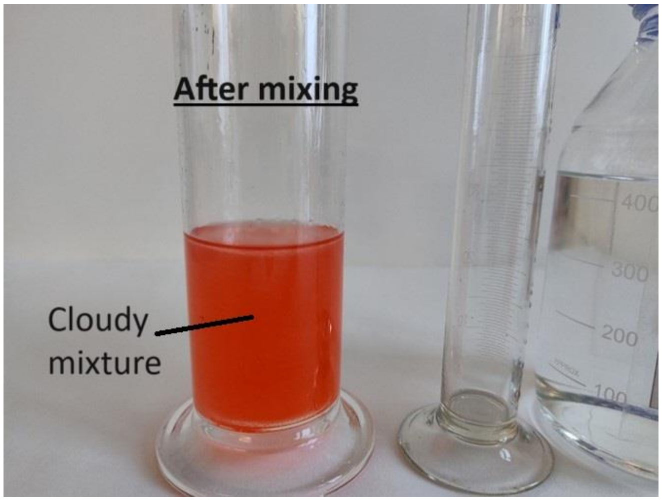


Figure 2. Complete set of tubes used to test the solubility in water after the corresponding alcohols were added.

A decrease in the solubility appears clearly between 1-propanol and 1-butanol. For the latter, when mixing both compounds, a milky mixture is easily seen (Figure 3A). Shortly after, a biphasic mixture begins to appear (Figure 3B).

A



B

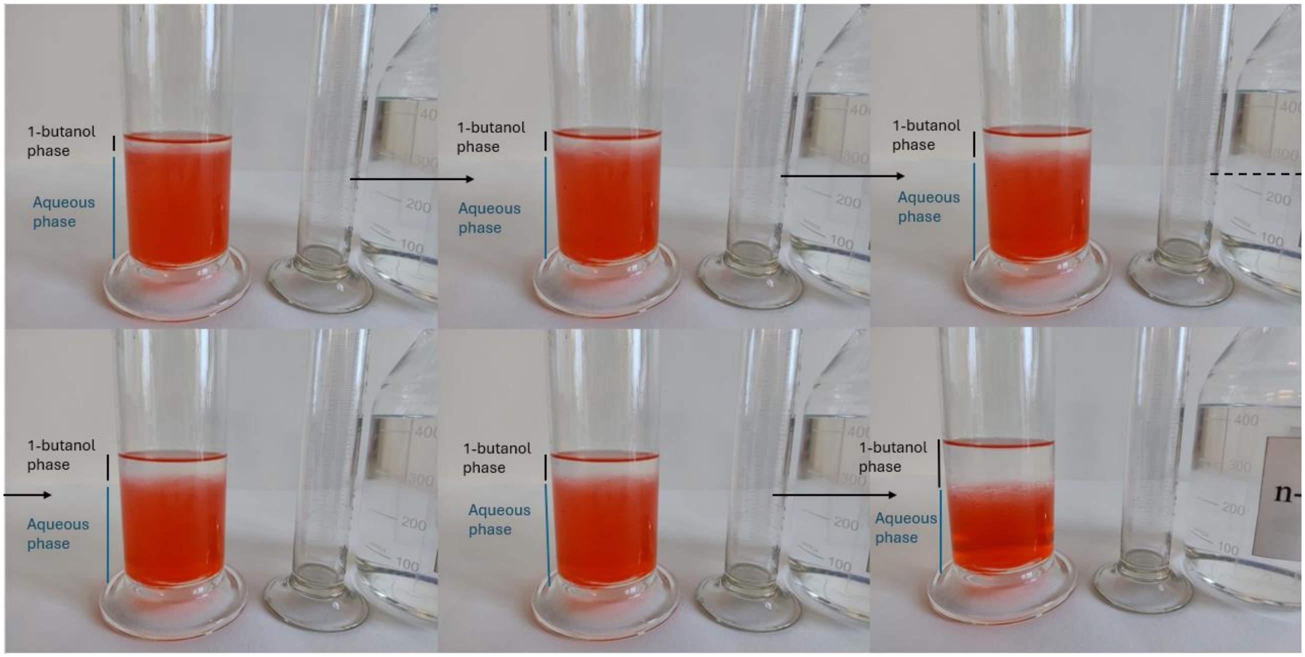


Figure 3. Mixing water and 1-butanol. After shaking the mixture, a milky aspect is observed (A); then, a few minutes later, a biphasic mixture appears (B) (first row from the left to the right and then second row from the left to the right).

In the Supporting Information, the results obtained from the assays involving the polyhydroxylated compounds are presented to show that although the carbon side chain increases, the presence of numerous polar hydroxyl groups induces a high solubility in water due to a high polar/nonpolar groups ratio (Figure S5).

1.2. The presence of H bonds

Mixing methanol, or ethanol, and water leads to an increase in the temperature of the mixture (Figure 4). This is due to the redistribution of hydrogen bonds between both compounds. During these live assays, the students are invited to touch the flask to note the variation of temperature, which can be easily detected manually (see Figure S6 in Supporting Information).

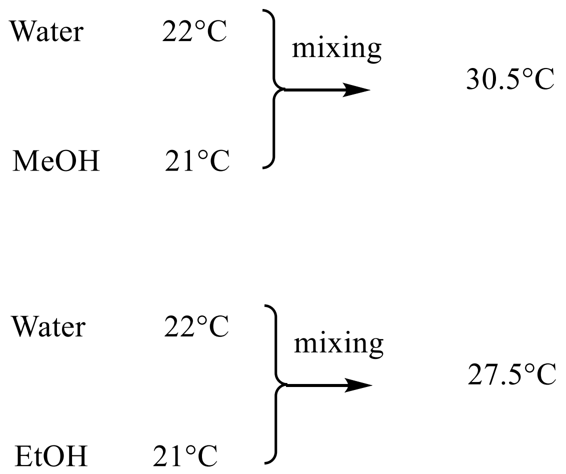


Figure 4. Addition of methanol or ethanol in water induces an increase in temperature.

In addition to these first experiments (Figure 2), sodium chloride is used as a competitor to show the involvement of hydrogen bonds. Sodium chloride is easily soluble in water due to its dissociation into ions (Na^+ and Cl^-) that are then solvated by interacting with water through hydrogen bonding. Thus, 1-propanol was added to a 50% saturated aqueous solution of sodium chloride. Contrary to what was seen when mixed with water, after stirring, a biphasic mixture appears (Figure 5). Based on the salting-out effect, the lipophilic character of the propyl side chain is shown. This approach might be used as a green approach for extraction.[10]

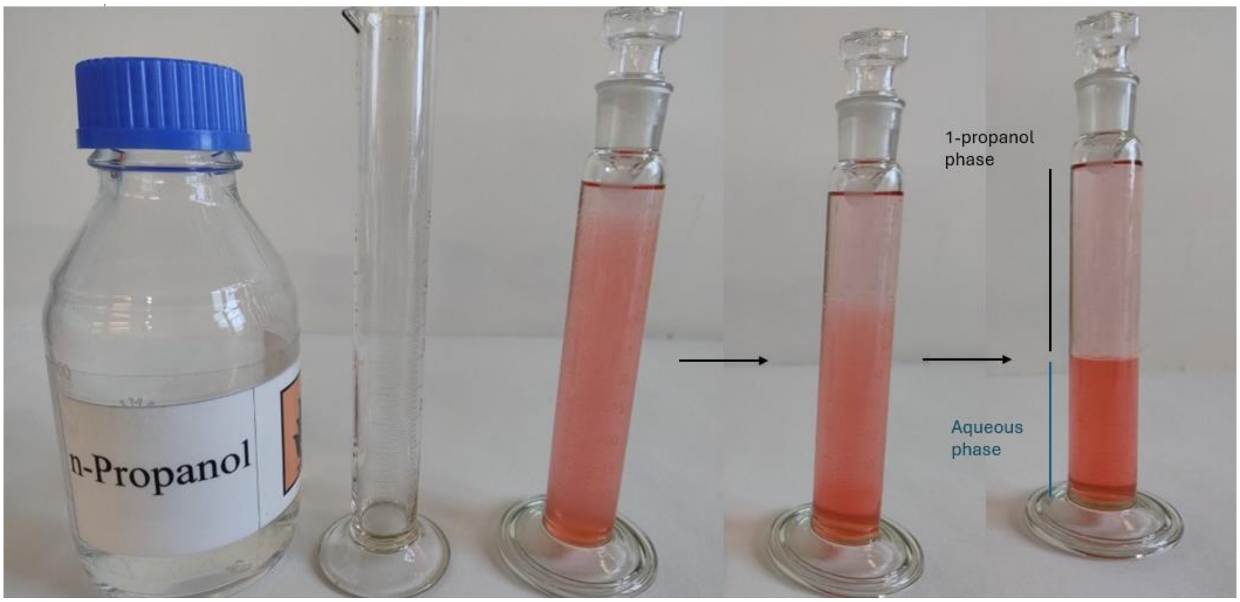


Figure 5. Mixing 1-propanol and the NaCl solution leads to a biphasic mixture, which progressively appears.

1.3. The mobile hydrogen or dissociation

In these assays, common carboxylic acids, such as acetic acid, a hydrophilic acid (transparent liquid), and benzoic acid, a lipophilic acid (white powder), are used. A discussion and an assay are also conducted with the corresponding conjugate bases (sodium acetate and sodium benzoate). These last compounds are ionic entities characterized as white powders and are hydrophilic. The assays involving acetic acid and sodium salt are not performed during the demonstration (see Supporting Information Figure S7).

For benzoic acid and sodium benzoate, the difference in terms of water solubility is significant. After the addition in water and mixing, benzoic acid is not solubilized due its lipophilicity (Figure 6B-C), while the sodium salt is easily soluble and gives a transparent solution (Figure 6B-C).

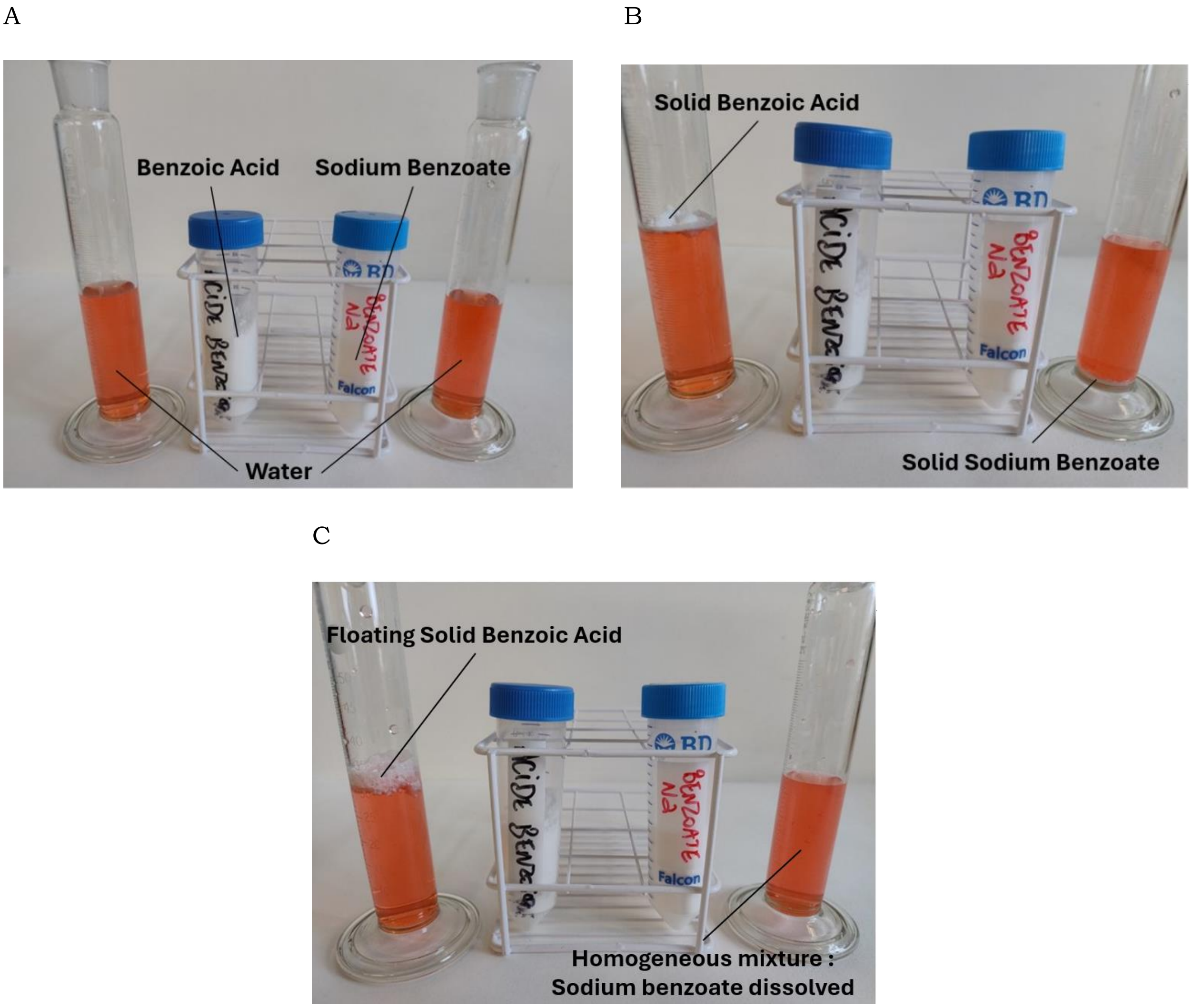


Figure 6. Assay demonstrating the solubility of benzoic acid and its conjugate base (sodium benzoate) in water. (A) Tubes before addition. (B) Tubes immediately after addition. (C) Tubes a couple of minutes after mixing.

2. The potential ionization when solubilized in water

To further understand the process of solubility, the ionization phenomenon is demonstrated using the results obtained from the conductivity measurements. It is known that hydroxyl groups have a mobile hydrogen and are the acid form of the acid/base couple chemically speaking. Students are familiar with the pKa table, but they must learn to take into account the context. The following assays use an aqueous medium partially or totally and will allow a deeper understanding of the difference between the various hydroxylated compounds. Unlike a weak acid, a strong acid is completely deprotonated in water following an acid/base reaction, and ions will appear in the solution. On the other hand, the corresponding sodium salts are ionic entities, hydrophilic, and easily soluble in water.

Aqueous solubility is obtained by forming hydrogen bonds, as shown above, but also by the ionization or the dissociation of certain functional groups. The protonation and deprotonation phenomenon is a classical mechanism in organic chemistry. Here, the strength of the corresponding acid or base must be examined in the context of aqueous media.

In these experiments, conductivity measurements are used because they are a reliable way of measuring the presence of ions in a solution. It is known that water purification systems are controlled by the continuous measurement of its conductivity. Due to the absence of ions, the conductivity is null, while the resistance is very high, around 18 MΩ. Since these measurements take time, the results alone are presented during the live demonstration.

For the first assay, aliquots of saturated aqueous NaCl solution are added to a volume of demineralized water and the conductivity is measured. Demineralized water had no conductance. Following the addition of NaCl in the demineralized water, a current appears due to the presence of ions (Figure 7).

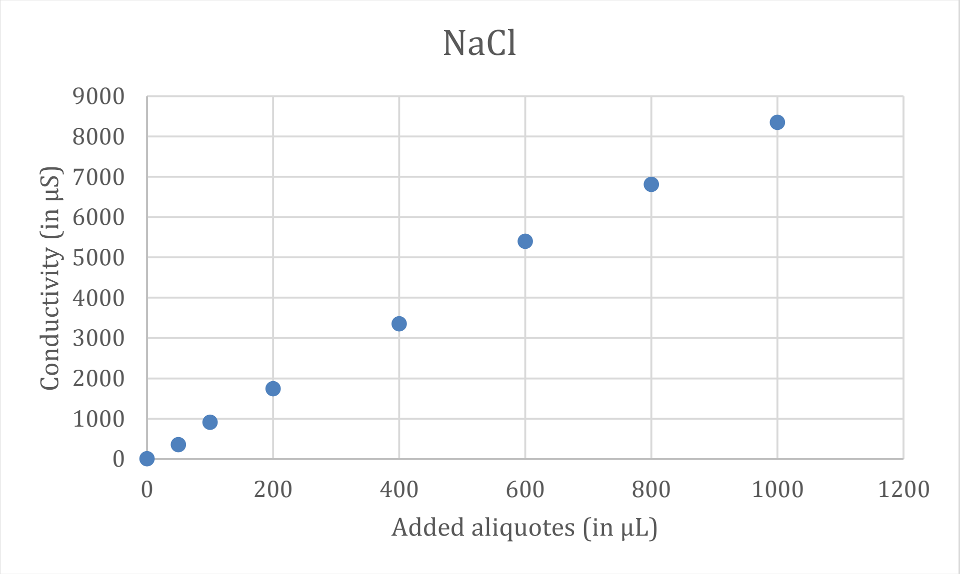


Figure 7. Addition of NaCl in demineralized water leads to an increase in the conductivity.

With ethanol (and a polyhydroxylated compound), no current occurs (Figure 8), meaning no ionization. This means that the alcohols with a short-carbon chain are easily soluble in water only by the presence of intermolecular interactions such as H bonds. The aliphatic hydroxyl group is not acidic enough to induce an acid/base reaction with water. In this condition, the proton is not mobile enough. This will also be confirmed later in the demonstration.

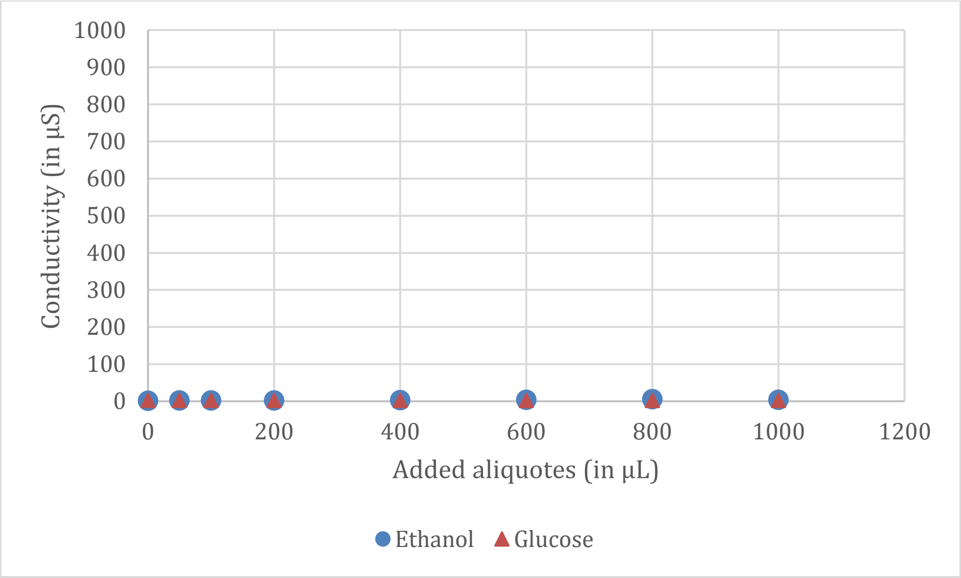


Figure 8. Addition of ethanol and a polyhydroxylated compound (glucose) in demineralized water has no effect on conductivity.

With acetic acid, a current is observed (Figure 9). In parallel to the hydrophilicity due to the short-carbon chain and the polarity of the functional carboxylic group, an ionization is observed due to the acid-base reaction with water (Scheme 1). The fact that the acid is hydrophilic facilitates this reaction. In the same graph, the result obtained with the conjugate base (sodium acetate) shows that this compound is ionized (Figure 9).

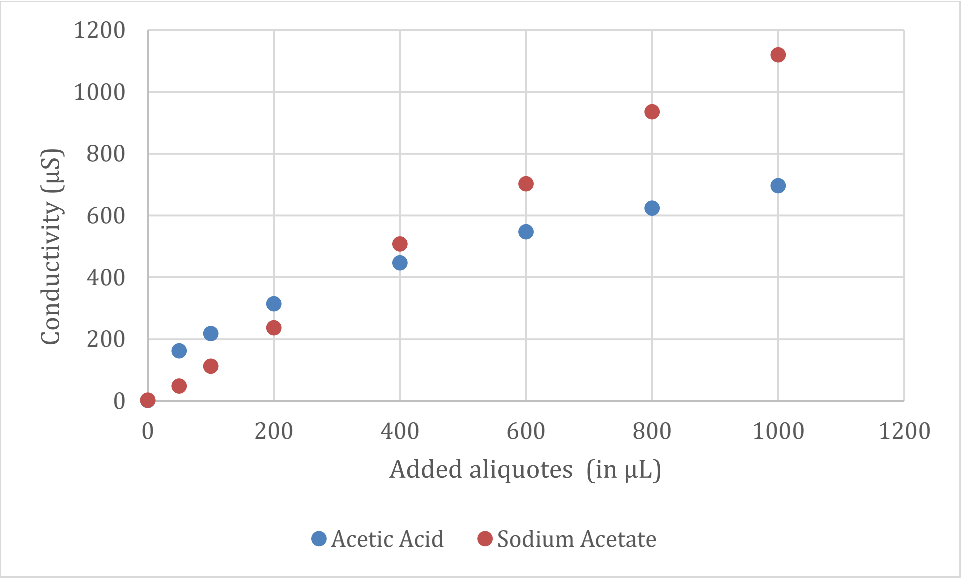
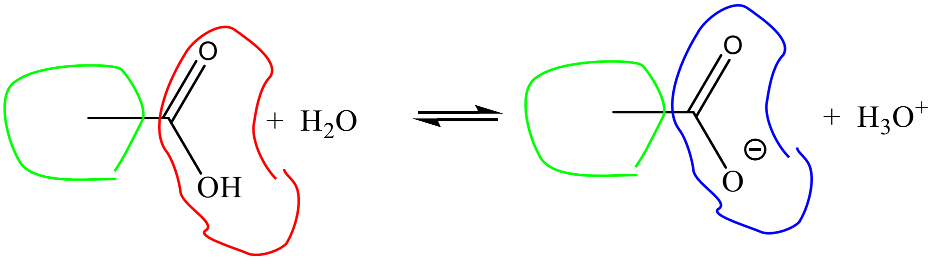


Figure 9. Addition of acetic acid and sodium acetate in demineralized water increases the conductivity.



Scheme 1. Acid/base reaction between acetic acid and water leads to ionic entities (in red, the polar group interacting by H bonds; in blue, the polar group interacting by ionic bonds; in green, the hydrophobic nonpolar group).

The addition of triethylamine, a small basic compound, to demineralized water leads to an increase in conductivity (Figure 10). This tertiary amine reacts with water following an acid/base reaction (Scheme 2).

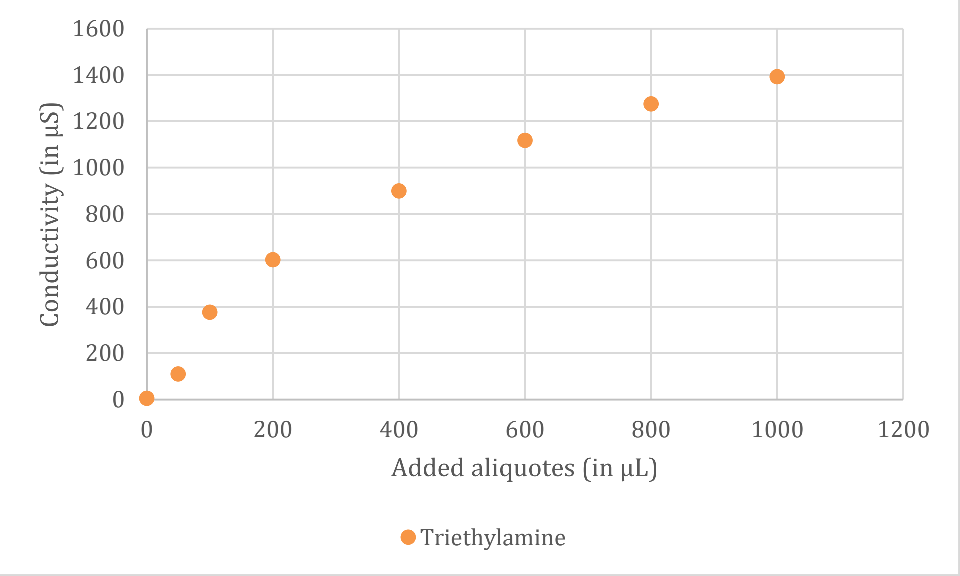
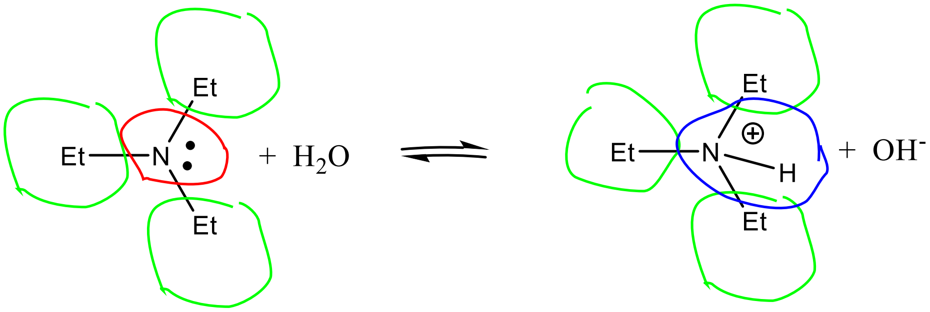


Figure 10. Addition of triethylamine in demineralized water increases the conductivity.



Scheme 2. Acid/base reaction of triethylamine with water (in red, the polar group interacting by H bonds; in blue, the polar group interacting by ionic bonds; in green, the hydrophobic nonpolar group).

The dissolution of this weak lipophilic amine in water followed by the acid/base reaction with water generates an excess of hydroxide ion, which renders the medium basic (Scheme 2), while the amine group is protonated. This reaction is quite easy with hydrophilic amine, and the reaction will be limited when the lipophilicity of the compound increases.

Then, the result obtained with a quaternary ammonium derivative, tetra-*n*-butylammonium hydroxide, is presented (Figure 11). It is a permanently charged nitrogen group. The addition of the compound to demineralized water induces a high impact on the conductivity of the solution.

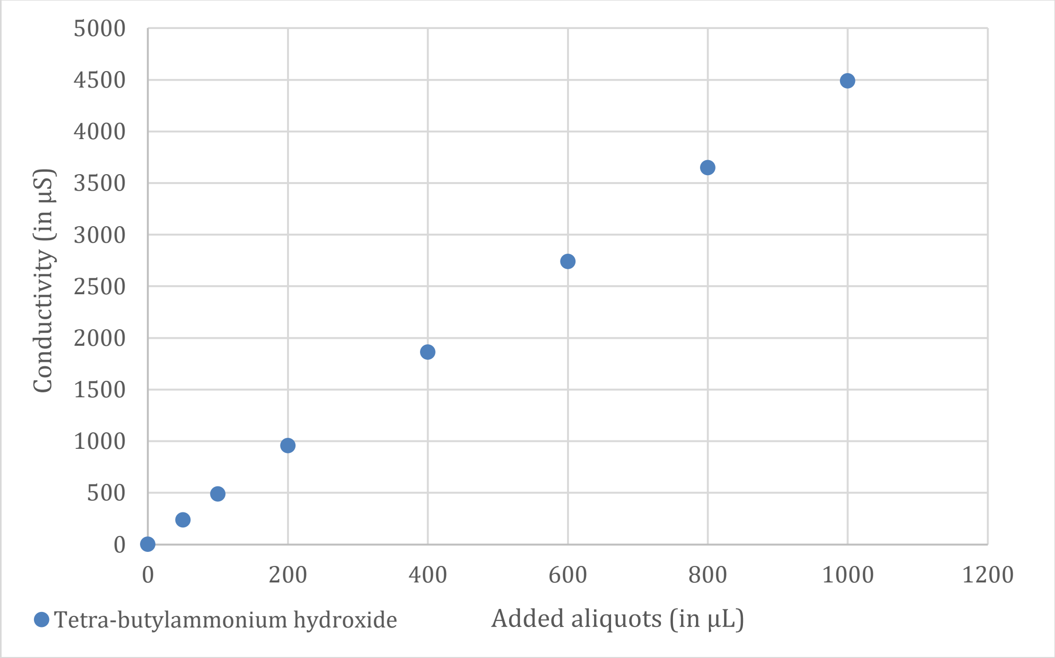


Figure 11. Zffect of tetra-*n*-butylammonium hydroxide on the conductivity of demineralized water.

3. EXTRACTABILITY

During the live demonstration, the results of the experiments are presented, and the liquid-liquid extraction procedure is described (see Supporting Information). The students have learned and used this technique during the practical sessions of their organic chemistry course.

Here, the extraction corresponds to the transfer of the product from the aqueous phases, which are buffered solutions at different values of pH, to the diethyl ether phase. Therefore, in these experiments, the nonionized fraction of the lipophilic compound is extractable and will be found in the organic phase.

To introduce the set of extractability experiments, a test, using an aqueous solution of sodium benzoate (Figure 12A), was shown to demonstrate what happens to a salt when the pH is changed. In one tube, a few drops of aqueous 3 N HCl are added. Immediately after, a white precipitate of benzoic acid appears (Figure 12B) due to the lipophilic behavior of the compound in its acid form. The protonated form is uncharged and lipophilic, while the deprotonated form, the conjugate base, is charged and hydrophilic (Scheme 3).

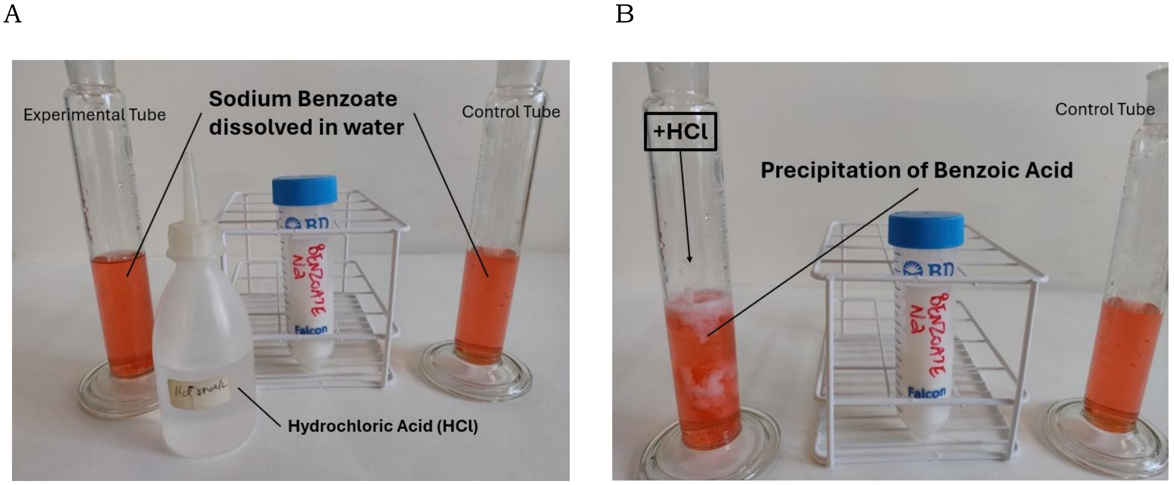
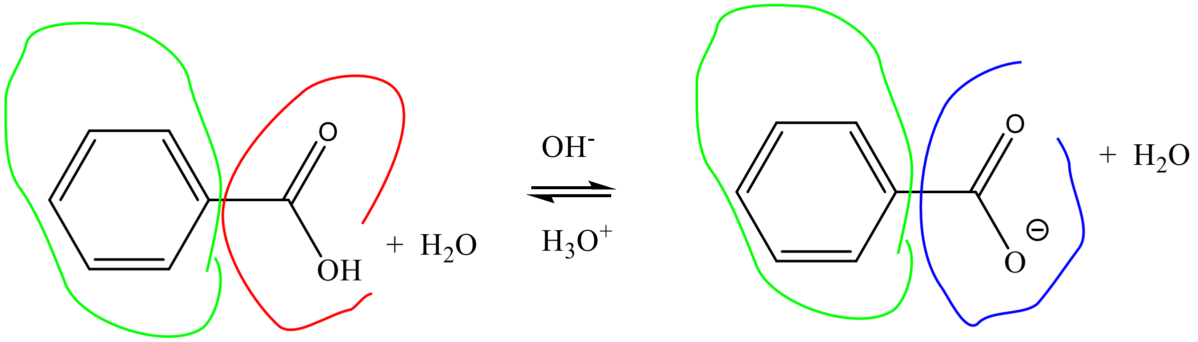


Figure 12. Assay with sodium benzoate (conjugate base of benzoic acid) and variation of pH. (A) Before the addition of HCl. (B) After the addition of HCl.



Scheme 3. Influence of pH on either the acid form (benzoic acid on the left) or the conjugate base (sodium benzoate on the right) (in red, the polar group interacting by H bonds; in blue, the polar group interacting by ionic bonds; in green, the hydrophobic nonpolar group).

This indicates that the hydrophilic/lipophilic properties of a compound can be pH-dependent. In this experiment, the carboxylate (conjugate base) is protonated to form the carboxylic group which is now nonionized and induces the precipitation of the molecule due to the lipophilic character of the molecule (Figure 12B-13A). Furthermore, the addition of an organic solvent (chloroform or diethyl ether) to this tube leads to the nonsoluble benzoic acid being dissolved by the organic solvent, and the biphasic mixture once stirred is completely transparent (Figure 13A-C). Similar assays were performed for each of the extraction experiments. The original aspect of this experiment is to clearly visualize the extractability process by evaporating the solvent after extraction at each of the pH values in order to quantify the compound extracted.

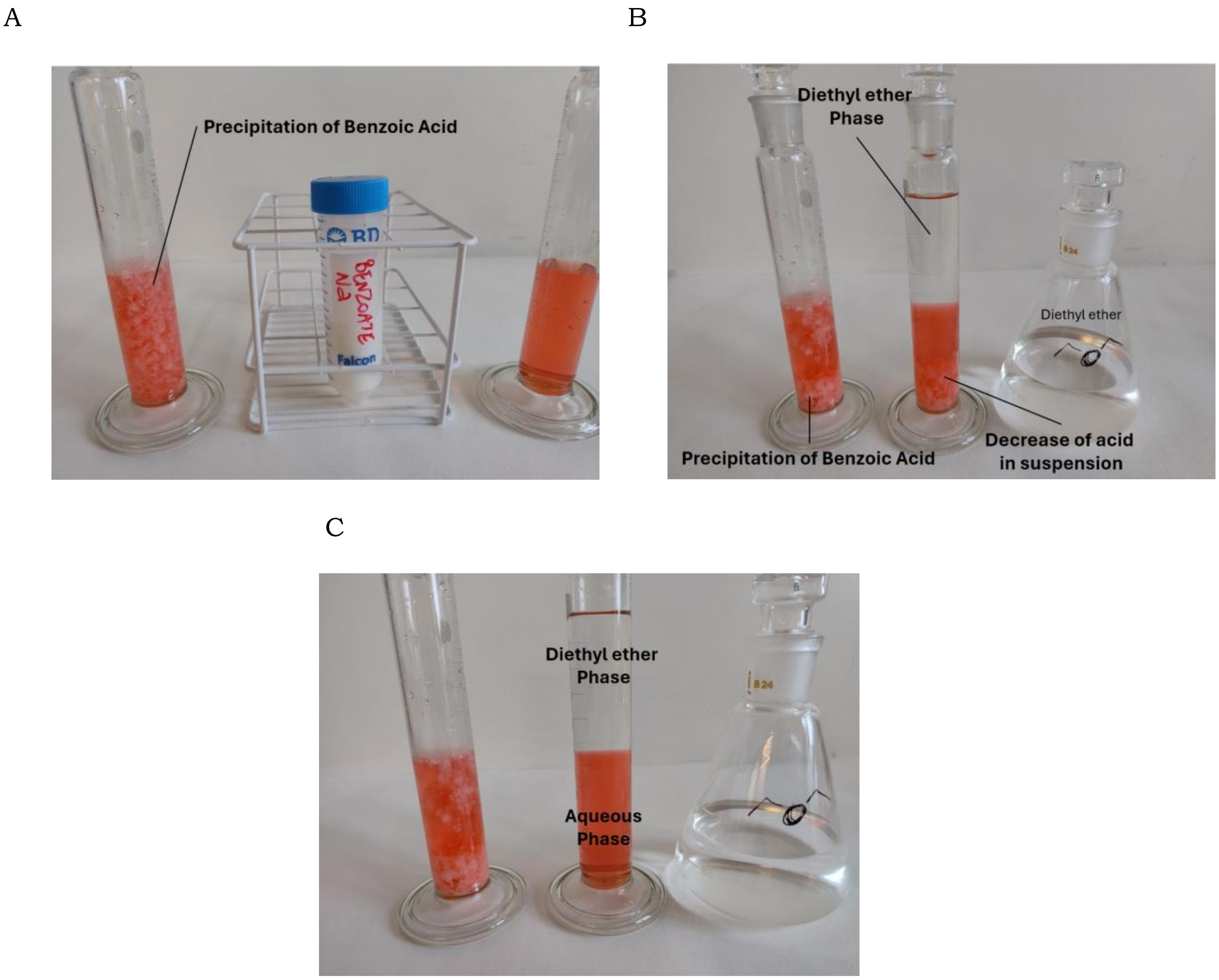


Figure 13. Assay showing the extractability concept. (A) After the addition of HCl, (B) after the addition of diethyl ether, and (C) after the mixing of both phases. The precipitate of benzoic acid is extracted by the organic solvent and disappears from the aqueous phase.

In this demonstration, compounds possessing a hydroxyl group or related, and one amine were tested (Figure 14). For practical reasons, nonionized forms are lipophilic and solid. Indeed, it was shown that small size molecules are easily soluble in water and their extractability was strongly hampered.

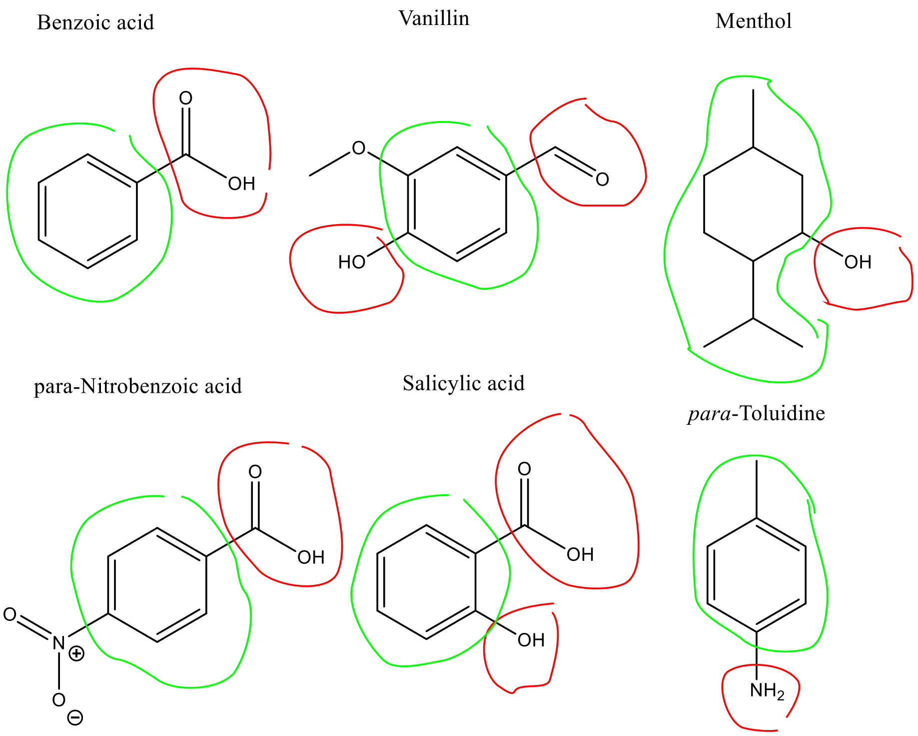


Figure 14. Chemical structures of compounds used for the extraction assays (in red, the polar group interacting by H bonds; in green, the hydrophobic nonpolar group).

The experiments give the anticipated curves, which are presented and discussed. For the carboxylic acid derivatives and phenol derivatives, each extractability curve resembles a sigmoid-like curve (Figure 15-17). As a message in the context of the isolation of compounds, to fully recover the corresponding compound in the organic phase, it is necessary to fix the pH to a lower value than the pKa of the compound (for an amine, the pH will be fixed to a higher value than the pKa). For menthol, a lipophilic alcohol, the curve is linear as the compound is always present in the organic phase (Figure 18). The alcohol group (pKa ~ 15-18) is considered as a neutral entity in the pharmaceutical and biomedical field. In water, it will not be deprotonated unlike the carboxylic acid (pKa ~ 4-6) and the phenol (pKa ~10-11) analogs. Regarding the amine, here a primary aromatic one (pKa ~ 4-5, corresponding to the protonated form; see Scheme 2) (Figure 19), the sigmoid-like curve is reversed compared to the acid compounds. To recover the corresponding compound in the organic phase, it is necessary to fix the pH to a higher value than the pKa of the compound.

The results of the extractability assays show that the carboxylic acid is a stronger acid compared to the phenol or the phenol is a weaker acid compared to the carboxylic acid, as it can be deduced from theoretical pKa values. It is thus clearly shown that a hydroxyl group presents a different behavior when attached to an aliphatic moiety, as opposed to an aromatic ring (Figure 17 and 18). The pKa is effectively lower for the phenol compared to the alcohol, and it is possible to deprotonate the phenol in aqueous conditions.

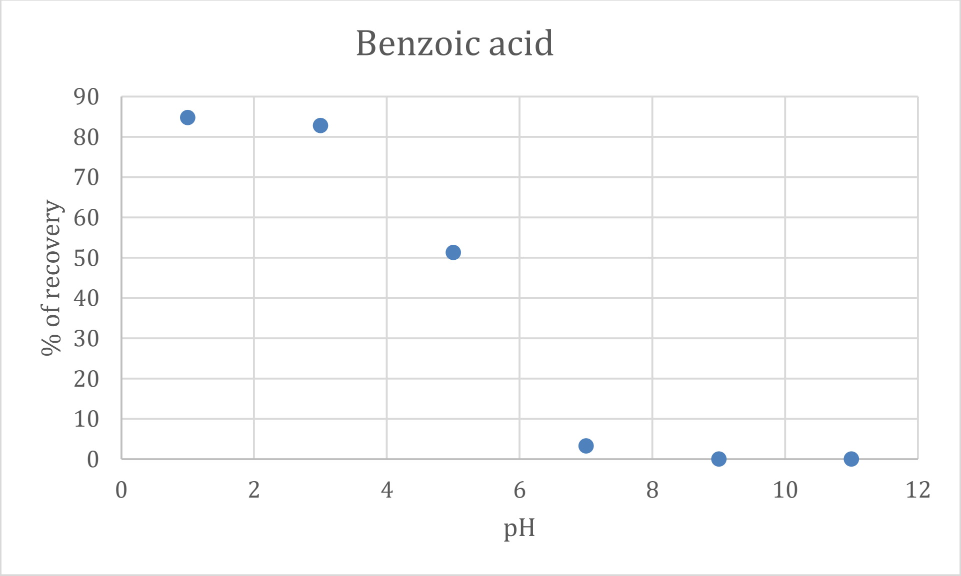


Figure 15. Benzoic acid extractability curve.

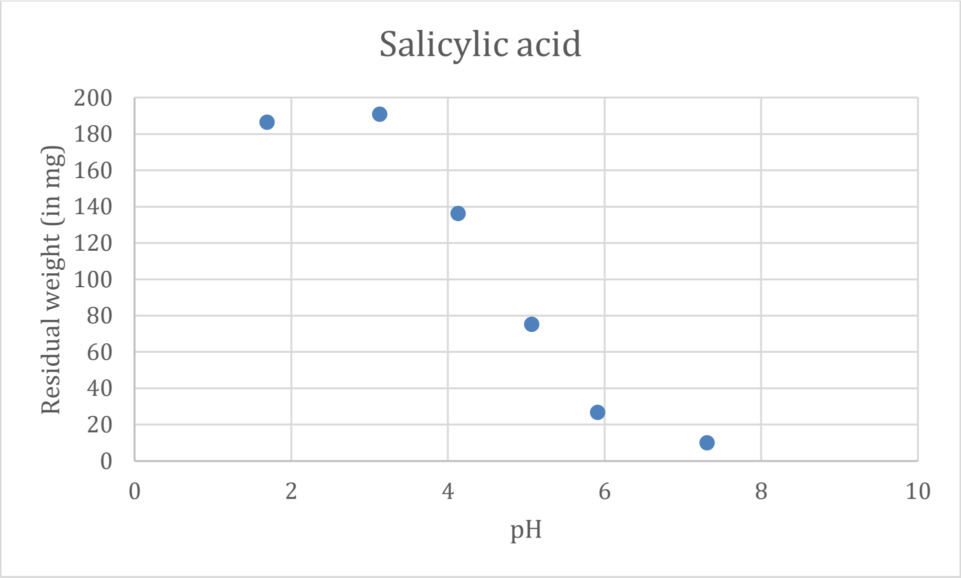


Figure 16. Salicylic acid extractability curve.

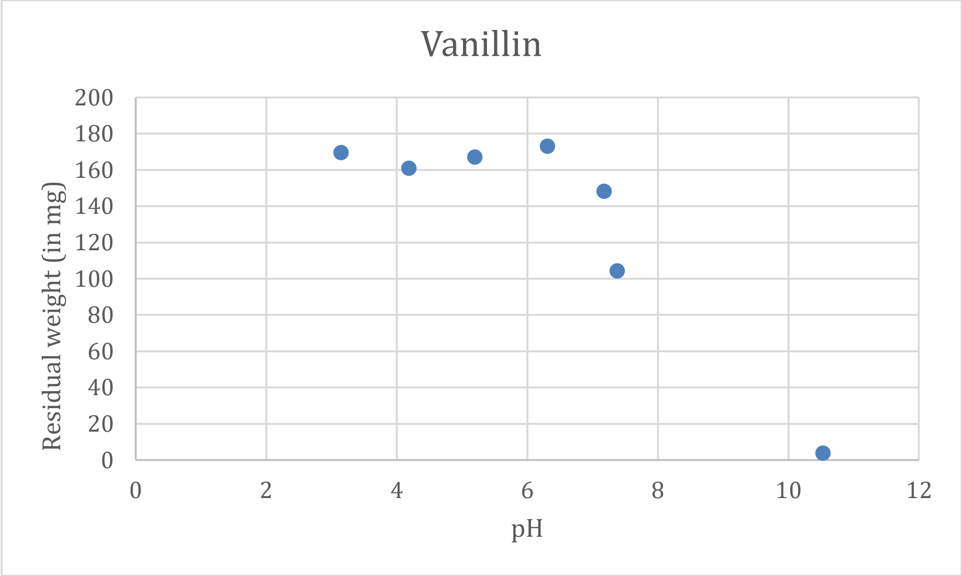


Figure 17. Vanillin extractability curve.

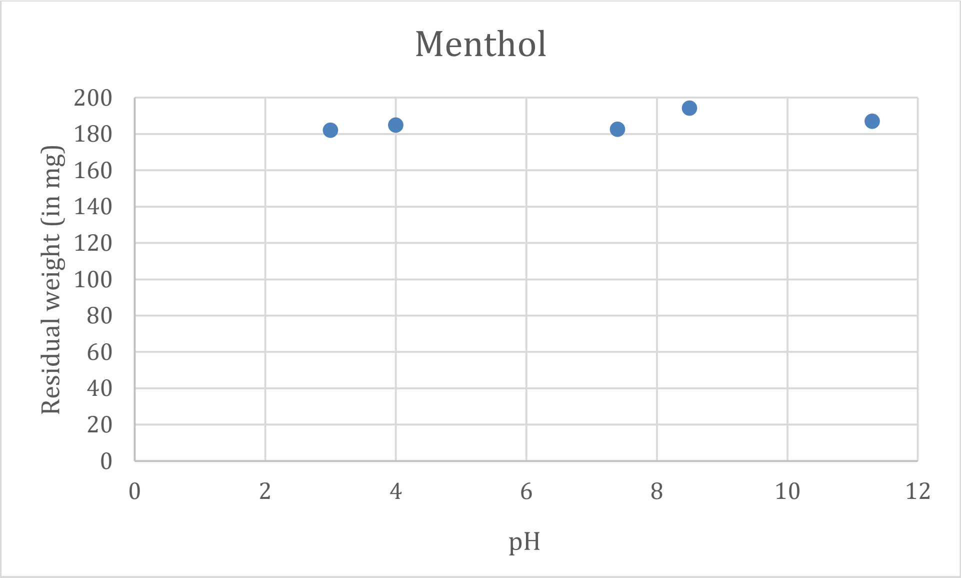


Figure 18. Menthol extractability curve.

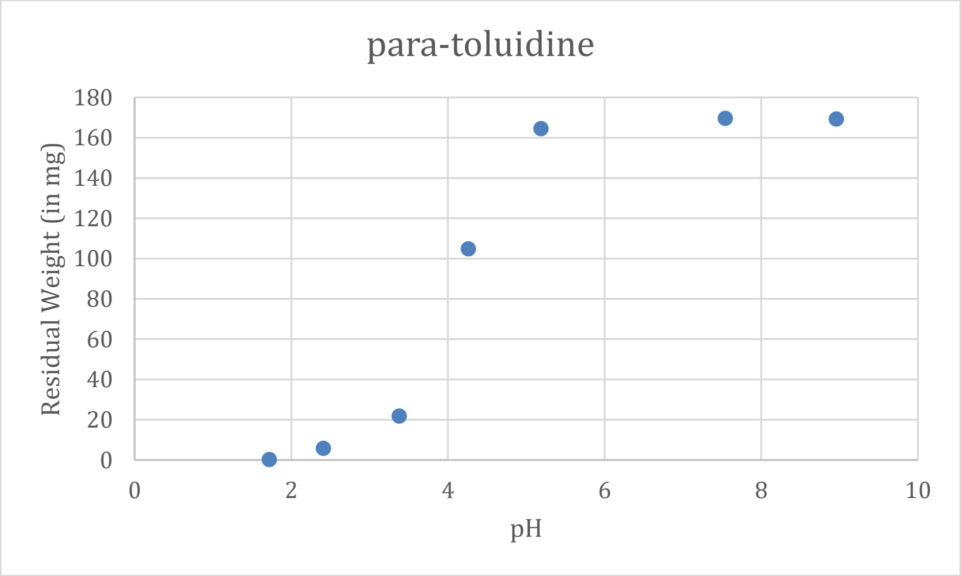
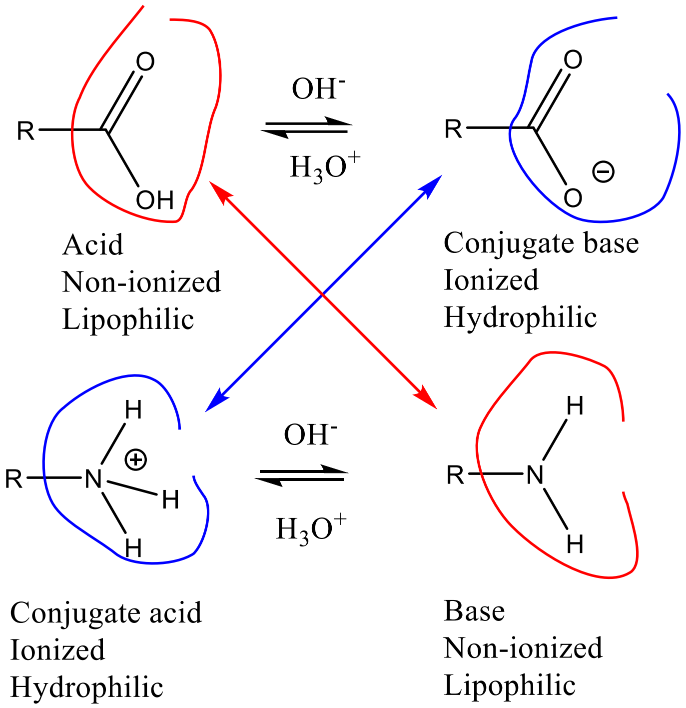


Figure 19. *para*-Toluidine extractability curve.

With such results, it is possible to generate theoretical curves of compounds based on the acid/base character of their specific functional groups. The nonionized form of the compound is lipophilic (depending on the carbon side chain) and will be found in the organic phase, while the ionized form is hydrophilic and will be present in the aqueous phase. This relationship (Scheme 4) will enable the extractability curves for the corresponding molecules to be drawn (Figure 20). Without going into details concerning the pKa of functional groups and their involvement in the extractability process, it is possible to see that there is a relationship between the curve at 50 % (weight or % of recovery) and the pKa value. Otherwise, it is important to mention that the curves for acidic compounds and basic compounds are shifted to either the right or the left depending on the force of the acid or the base.

This concept and the curves are extensively explained during the course and the seminars and asked during examinations (see Supporting Information).



Scheme 4. Cross relationship between ionized and nonionized entities in lipophilic carboxylic acid and amine (in red, the polar group interacting by H bonds; in blue, the polar group interacting by ionic bonds). This will lead to different extractability profiles.

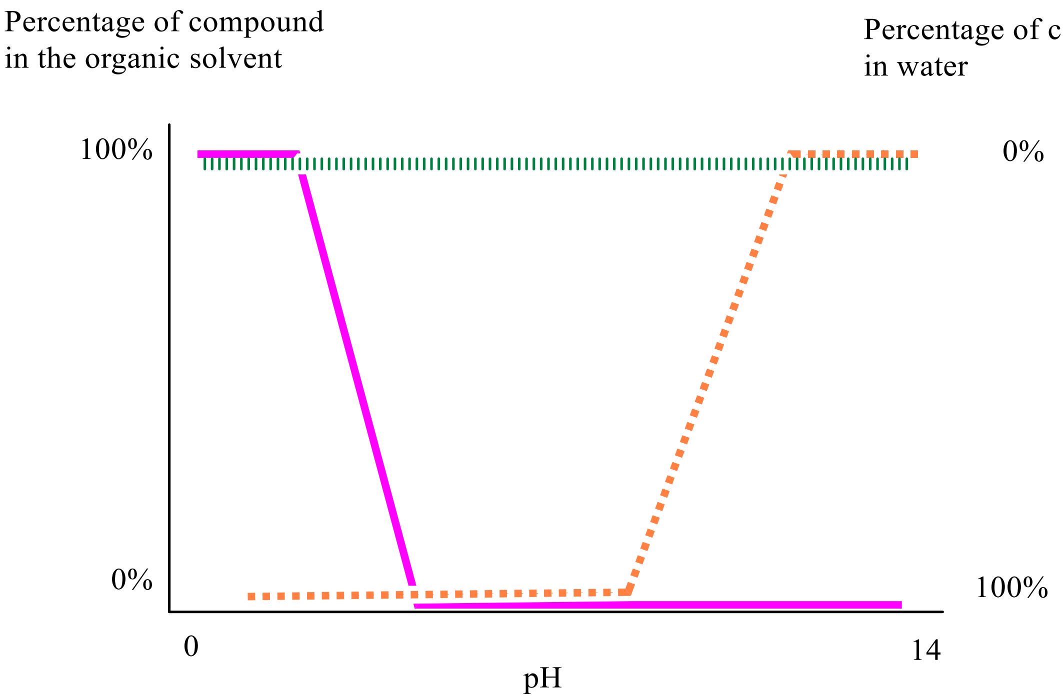


Figure 20. Theoretical extractability curves for the acid compound or its conjugate base (full line), the base compound or its conjugate acid (dashed line), and the lipophilic neutral compound (hashed line).

In conclusion, these simple assays addressed to undergraduate students may help them understand the phenomena involved in the solubility and extractability of organic compounds. Different learning outcomes are expected (Table 1).

Table 1. Learning outcomes of this live demonstration

Visualizing the solubility of liquid and solid compounds in water and their limitation by lipophilic character.	Part I
Understanding the solubility of liquid and solid compounds through their H bond interactions and their interaction with water.	Part I
Visualizing the solubility of liquid and solid compounds in water due to their ionization.	Part II
Showing that a hydroxyl group has a different acid behavior depending on the electronic environment within the functional group (alcohol, phenol, carboxylic acid).	Part II-III

Showing the difference in the acid character of different functional groups.	Part III
Showing the differential behavior between an acid (or base) and their conjugate form in terms of solubility and extractability.	Part III

These concepts are extensively utilized by the students throughout their practical sessions as they study unknown compounds. In the continuation of the course curriculum, this demonstration will help explain the absorption, metabolism, and toxicity of different drugs. Indeed, the various absorption pathways (skin, gastrointestinal tract, pulmonary tract, etc.) have different pH levels, and the absorption by passive diffusion is partly explained by the behavior of molecules depending on the pH. Furthermore, with the experience of this live demonstration, a practical session related to the extractability of a larger panel of pure compounds was recently designed and successfully introduced in the program of practical sessions.[11]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.XXXXXXX. [ACS will fill this in.] It contains the materials and reagents, the different protocols, the timing of the live demonstration and some additional assays, report of a practical session in relation to this demonstration and question of examinations, the video with English subtitles. (PDF)

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ACKNOWLEDGMENTS

A. Bouillez is gratefully acknowledged for the technical assistance in preparing the experiments. Z. Taouba is gratefully acknowledged for the careful reading of the manuscript. JFL, HT and JLH have a Master’s degree in Pharmacy. HT is an assistant at the Department of Pharmacy of ULiège. JLH is starting a PhD thesis as Research Fellow of the F.R.S.-FNRS. JFL is a Research Director of the F.R.S.-FNRS and the professor of the course CHIM0714 at the ULiège for more than two decades.

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